CHAPTER 39

The Control of Microorganisms in Flowing Maple Sap by Ultraviolet Irradiation

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Growth of adventitious microorganisms in maple sap causes deterioration and results in the production of low grade syrups. A study was conducted to determine the suitability of the germicidal rays of ultraviolet light to control the growth of these organisms. The use of these rays eliminated the objections associated with the use of chemical germicides since they produced no residues nor had any deleterious effect on the delicate maple flavor. This study also showed that the ultraviolet irradiation method permitted the continuous treatment of flowing sap with a 99% kill of the organisms.

The control of microbial contamination of maple sap has long been recognized as a prerequisite to the production of high quality maple syrup (Costilow et al., 1962; Naghski and Willits, 1953; Wasserman, 1963; Willits and Sipple, 1961). Maple sap is readily degraded by microorganisms in such ways that syrup made from contaminated sap is dark colored and may have "off" flavors and/or a poor texture (ropiness) (Wasserman, 1963). Raw maple sap is naturally sterile; but in its collection and handling, it is subject to contamination. The sap producer and evaporator operator are now recognizing that contamination and the resulting microbial activity in the sap must be controlled to minimize sap spoilage. In the past, the only means of keeping this activity in sap to a minimum was to avoid its storage for periods beyond 24 hours. In recent years, the use of germicidal taphole pellets (Costilow et al., 1962), plastic bags (Naghski and Willits, 1953), and plastic tubing (Willits and Sipple, 1961), and the improvement of sanitary procedures (Wasserman, 1963) have enabled sap producers to decrease losses from microbial spoilage. These have not contributed materially to increasing sap storage time. To increase this safe storage period requires either the complete sterlization of the sap or the control of the microbial population by keeping it at such a low level that any fermentations that take place before processing will be minimal.

In 1953, Naghski and Willits reported on the germicidal effect of solar ultraviolet irradiation on maple sap collected in transparent plastic bags. Further investigations on irradiation of maple sap using sunlight and ultraviolet lamps, in which wave lengths known to be lethal to microorganisms (260 to 270 mμ) were used, were carried out by Frank and Willits (1960) and Schneider, Frank, and Willits (1960). The latter work, done on a laboratory scale, indicated that sap contaminants could be effectively reduced by the use of ultraviolet irradiation, and also showed that the sap could be effectively irradiated using ultraviolet lamps of the types used for irradiation of cane sugar solutions and home water supplies.

Ultraviolet irradiation has been used for many years in the sterilization of water

(Bottolph, 1955). Since this is a physical method of sterilization, it would appear to be ideally suited to the sterilization or pasteurization of maple sap, because ultraviolet radiation does not have any harmful effect on the delicate maple flavor in the syrup. It causes no change in either sap-contained flavor precursors or the maple flavor in the resulting syrup. It also eliminates the possibility of off-flavors owing to side reactions, which could occur if a chemical disinfectant or bacteriostatic compound were to be used to effect the microbial control. Sap is a water solution containing only 1-4% dissolved solids which are mostly sugars. It is comparable to water in clarity, transmission of ultraviolet radiation, and flow characteristics. Thus, it is an ideal medium for irradiation with ultraviolet light.

The current practice is to merely make a coarse filtration of the sap to remove leaves, bark, and other foreign matter prior to its storage. A recent study (Kissinger, Sipple, and Willits, 1964) showed that the average bacteria counts in sap ranged from 2×10^4 to 33×10^4 organisms per ml. The use of ultraviolet water purification units, placed in the sap feed line between the filter and the storage tank, offered a good possibility for either sterilizing the sap or sufficiently reducing the bacteria counts to keep bacterial growth at a low level while the sap is held in the tank. This paper presents the results obtained by the ultraviolet irradiation of a flowing stream of contaminated sap.

Experimental

Apparatus

1) A 150-gallon galvanized iron tank, 2 ft wide x 6 ft long x 2 ft high.
2) A centrifugal pump producing a maximum flow of 10.5 gal per min through the ultraviolet light unit.
3) A valve in the pump discharge line to control flow rates.
4) A Fischer and Porter[1] flowrator, calibrated in 0.2 gal per min increments from 0 to 12 gal per min, to measure flow rates.
5) Ultraviolet light irradiation units, Aquafine model SP2. Each unit was equipped with two 30-watt ultraviolet lamps emitting in the region of 265 mμ (Costilow et al., 1962; Wasserman, 1963). Each lamp was mounted inside a 1-inch O.D. quartz tube, which, in turn, was mounted inside a 2-inch I.D. metal pipe with appropriate glands and fittings. In the process of irradiation, the sap flowed in a concentric layer ½ inch thick between the quartz tube and the metal pipe. The volume of the concentric layer of sap about the quartz tubes in the ultraviolet unit was 0.7 gal (0.35 gal per lamp). The time that the flowing sap was exposed to the germicidal rays is expressed by the equation:

$$\frac{\text{volume of sap in exposed area (gal)}}{\text{rate of flow (gal per min)}} = \text{exposure time}$$

The irradiation units were mounted in the discharge line in such a manner that the units could be used singly or in series.
6) All tubing lines were of Transtube plastic dairy hose.
7) Sap supplies
   a) Synthetic sap. Because of the unavailability of large volumes of maple sap, all large-scale experimentation was carried out with a synthetic maple sap made

[1] Mention of company or trade names does not imply endorsement by the Department of Agriculture over others not named.

by dilution of fancy grade maple syrup to 2.5° Brix with sterile water and the addition of 0.9 g per gal of sugar sand.

b) Sterile sap. Stored, frozen sap was thawed, sterilized by filtration through a membrane filter, and transferred aseptically to sterile flasks.

8) Microbial stock cultures

A mixed culture rich in *Pseudomonas, Leuconostoc, Bacillus* and other genera was secured from commercially produced sap. The mixed stock culture was maintained in sterile maple sap at 30 C and kept viable by transferring at 48-hr intervals.

The inoculum for the large volume studies was propagated by transferring 10 ml of a 48-hr culture to 150 ml of sterile sap in a Roux flask. This was incubated for 48 hr at 30 C, transferred to 1 gal of sterile synthetic sap, and incubated at 30 C for 48 hr. The cell concentration in the latter was determined microscopically using a hemacytometer. Based on this count, an amount of the above culture was added to the 150 gal of synthetic sap in the supply tank to yield a cell concentration of 1×10^5 per ml.

Two pure cultures of yeast were used in the irradiation studies. One, a *Saccharomyces* sp. identified by the laboratory code number 496-Y, was isolated from contaminated sap. The other was a *Saccharomyces* sp. isolated from the surface of a contaminated maple syrup and coded as 163-8. Yeast cultures were maintained on wort agar at 25 C and were transferred at 5-day intervals.

The inoculum for large volume sap studies was prepared by washing yeast cells with 10 ml sterile sap from a wort agar slant which had been incubated for 5 days at 25 C. The 10 ml of cell suspension was used to inoculate 150 ml of sterile sap in a Roux flask. This culture was incubated for 5 days at 25 C and then transferred to 1 gal of sterile synthetic sap. After 5 days of incubation at 25 C, the cell concentration was determined using a hemacytometer. Based on this count, an amount of the above culture was added to the 150 gal of synthetic sap to yield a cell concentration of 1×10^4 per ml.

9) Irradiation system

A flow diagram of the system is shown in Figure 1. The inoculated sap was pumped from the supply tank and successively passed through the flow control valve, flow meter, and irradiation unit or units and discharged.

10) Operation

The equipment was first sanitized by washing with 10% hypochlorite solution followed by 3 successive rinses with tap water. The tank was immediately filled with 140 gal of sterile synthetic sap and inoculated with the 48-hr culture of sap organisms. The sap was stirred to insure complete dispersion of the microorganisms

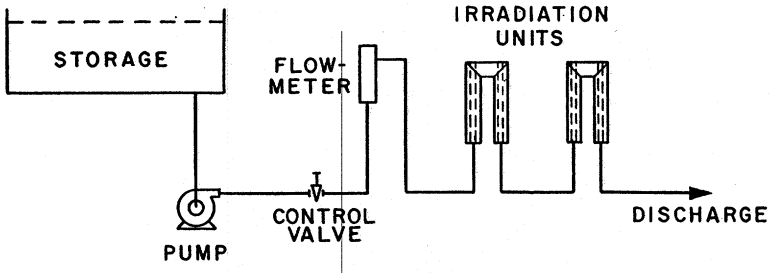

FIG. 1. Flow diagram of ultraviolet irradiation experimental system.

and then pumped through the system at rates of 5 to 10.5 gal per min, beginning with the slowest flow rate. This procedure was followed when either one or two units (2 or 4 ultraviolet lamps) were used.

11) Sampling and plating

Samples for bacteria counts were taken aseptically from the feed tank immediately after inoculation and mixing and from the discharge line. Following each change in flow rate, 2 min were allowed for equilibration before sampling from the discharge line.

Tryptone glucose extract agar (Difco) was used as the plating medium for bacteria counts. All plates were incubated at 30 C for 48 hr, and counts were made using a Quebec colony counter.

Wort agar (Difco) was used as the plating medium for yeast counts. All plates were incubated at 25 C for 5 days, and counts were made using a Quebec colony counter.

Results and Discussion

Preliminary studies were made to determine the lethal effect of the ultraviolet radiation produced by the lamps in the irradiation units. A flowing stream of the inoculated synthetic sap was irradiated at different exposure times varying from 8.4 sec to 42 sec using one irradiation unit and from 16.8 sec to 84 sec using two units. The different exposure times were obtained by changing the flow rates from 1 gal per min to 5 gal per min. The bacteria counts of the inoculated sap ranged from 1×10^3 to 1×10^5 organisms per ml. These studies showed less than 1% of the sap bacteria survived after the shortest irradiation exposure time, 8.4 sec. They also suggested that much shorter exposure times (more rapid flow rates) and/or increased bacterial concentrations were required to show significant differences in lethal effects of irradiation on sap bacteria.

Since the maximum flow rate was 1.5 gal per min, the shortest ultraviolet exposure time was limited to 4.0 sec when the single (2-lamp) unit was used and 8.0 sec for the double (4-lamp) units. Table 1 relates the irradiation time to the different flow rates through the single and double ultraviolet units.

Table 1. *Exposure times in single and double irradiation units for different flow rates*

Flow Rate Gal/Min	Irradiation Time, Seconds	
	Single Unit[a]	Double Unit[b]
5	8.4	16.8
6	7.0	14.0
7	6.0	12.0
8	5.25	10.5
9	4.7	9.4
10	4.2	8.4
10.5	4.0	8.0

[a] Commercial water irradiation unit containing 2 ultra-violet lamps.
[b] Commercial water irradiation unit containing 4 ultra-violet lamps.

The germicidal effects of irradiation of sap containing 1 x 10^5 bacteria per ml for the different flow rates (exposure time) in Table 1 are given in Figure 2. The irradiation caused decreases in bacterial count of 91% for 4.0 sec of exposure time and more than 99.5% for 8.4 sec of exposure. Figure 2 also shows that an exposure time of not less than 6 sec was required for a 99% reduction in the bacterial count. This indicates that the single unit cannot be used with 99% effectiveness with flow rates greater than 7 gal per min. However, for flow rates up to 10.5 gal per min, the two irradiation units used in series are effective. These curves show that the germicidal effect of the ultraviolet lamps is a function of exposure time. $\frac{\text{volume in exposed area (gal)}}{\text{flow rate (gal per min)}}$. Thus, the irradiation by the two units (4 lamps) produced the same effect, decrease in bacterial population, at 10 gal per min flow rate as obtained by one unit (2 lamps) at a flow of 5 gal per min.

The ultraviolet penetration of normal maple sap is the same as in water, with the transmission diminishing 1.5% per cm of depth. As the bacterial population in sap increases above 1 x 10^5, turbidity which develops decreases the ultraviolet transmittancy. To test the germicidal effect of the irradiation systems on cloudy (high bacterial count) sap, a synthetic sap with a bacterial count of 6.9 x 10^5 was used. The results of irradiation of this cloudy sap by single and double ultraviolet units are shown in Figure 3. Irradiation with the single unit resulted in only an 88% reduction of bacterial count at 4.0 sec exposure time (flow rate of 10.5 gal per min) and therefore was not considered satisfactory. However, doubling the exposure time by using the two irradiation units and maintaining the same flow rate caused a 99.2% reduction in bacteria count.

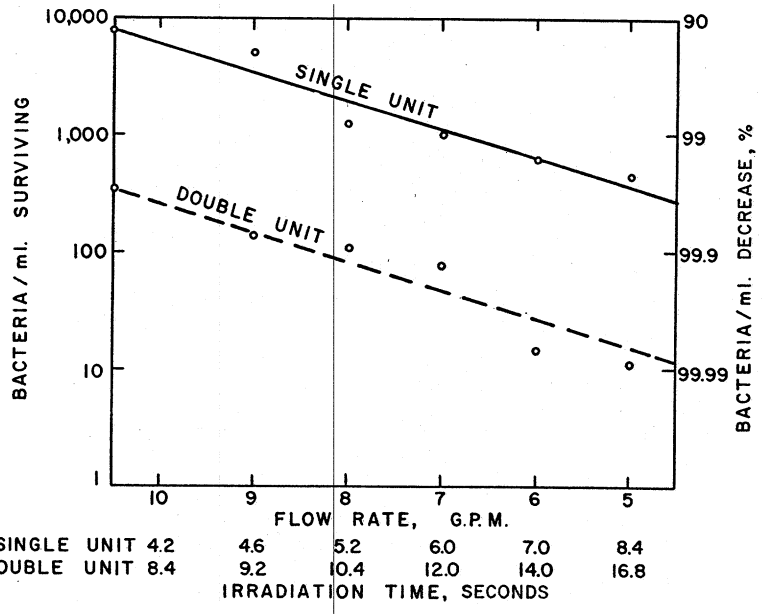

FIG. 2. Decrease in cell counts by ultraviolet irradiation of maple sap containing a cell count of 1 x 10^5 bacteria per ml of a mixed bacterial culture using single and double irradiation units.

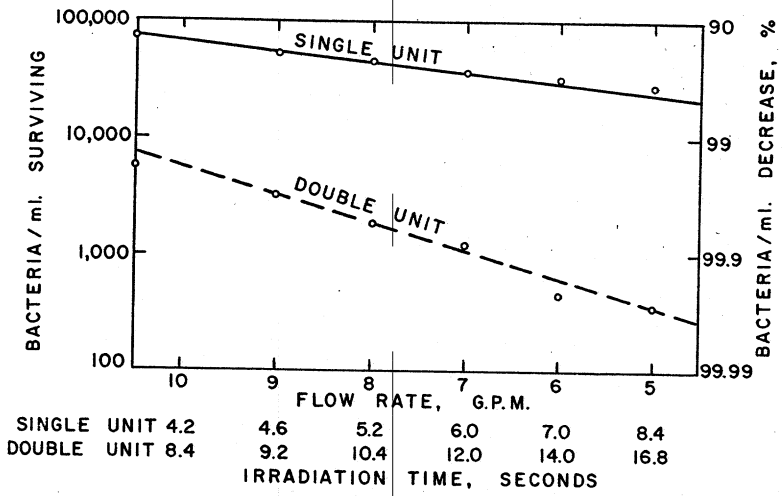

FIG. 3. Decrease in cell counts by ultraviolet irradiation of maple sap containing a cell count of 6.9 x 10^5 bacteria per ml of a mixed bacterial culture using single and double irradiation units.

Studies made on commercially produced sap showed that a yeast count of 1 x 10^4 colonies per ml represented the higher level of yeast contamination. To test the effectiveness of ultraviolet irradiation on the viability of yeast in maple sap, synthetic sap containing 1 x 10^4 cells per ml of yeast 496-Y was prepared. Irradiation of this sap at the different flow rates given in Table 1 resulted in decreased yeast counts as shown in Figure 4. An irradiation exposure time of 4.0 sec caused a 90% reduction in the yeast count and increasing the exposure to 16.8 sec reduced the yeast count by more than 99%. The curves in Figure 4 also show that exposure time of not less than 5.2 sec were required for a 99% decrease in yeast count and that flow rates in excess of

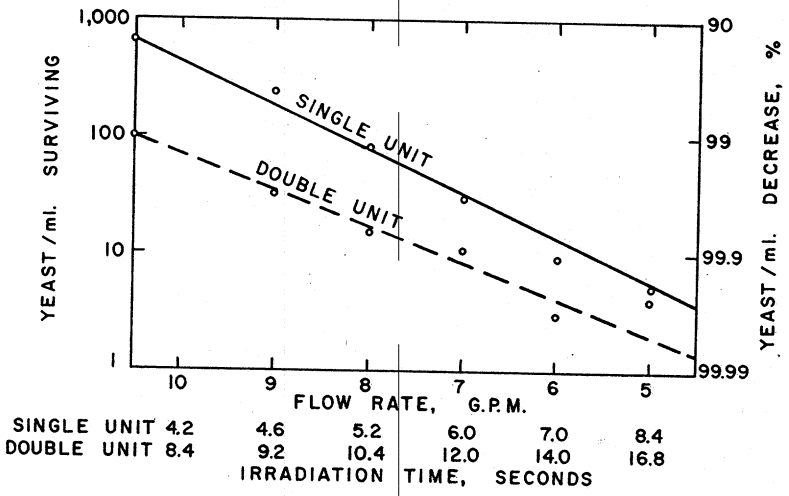

FIG. 4. Decrease in yeast cell count by ultraviolet irradiation of maple sap containing 1 x 10^4 cells per ml of a pure culture of No. 496-Y yeast using single and double irradiation units.

8 gal per min cannot be used with the single irradiation unit to attain this decrease in yeast population. But higher flow rates up to 10.5 gal per min can be effectively used with two irradiation units.

Yeast cells are larger than bacterial cells and require lesser numbers to produce turbidity. To test the germicidal effect of the ultraviolet irradiation systems on a cloudy (high yeast count) sap, a synthetic sap with a yeast count of 2 x 10^5 cells per ml of culture 163-8 was irradiated. Culture 163-8 was selected for this experiment because it produced a profuse growth in synthetic sap and a definite cloud occurred in the sap at the 2 x 10^5 cell concentration. The results of the irradiation using the single and double ultraviolet units are shown in Figure 5. The effectiveness of one unit at flow

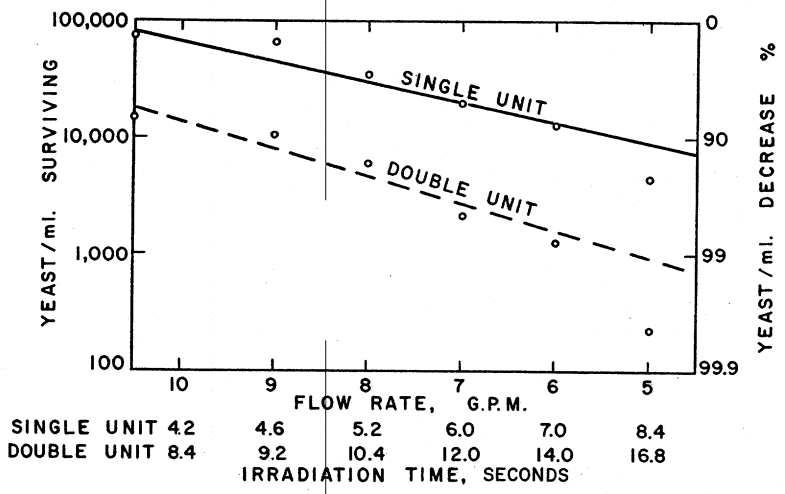

FIG. 5. Decrease in yeast cell count by ultraviolet irradiation of maple sap containing 2 x 10^5 cells per ml of a pure culture of No. 163-8 yeast using single and double irradiation units.

rates above 5 gal per min (exposure time 8.4 sec) was not satisfactory and increasing the flow to 10.5 gal per min (4 sec exposure) produced only a 55% kill of the yeast population. Irradiation of the same sap with the double ultraviolet unit resulted in a decrease of 99% in yeast population at flow rates of 7 gal per min (12.0 sec exposure) and above 90% at all flow rates. But the actual number of yeast cells surviving irradiation at a flow rate of 9 gal per min (9.2 sec exposure) was in excess of 1 x 10^4, the higher level of yeast contamination found in commercially produced sap. These results indicate that irradiation of sap containing a high yeast cell concentration is not as effective as irradiation of sap containing a high bacterial population. (Fig. 3). This is in agreement with the observations made by Schneider, Frank, and Willits (1960) in their studies of irradiation effects on pure cultures of bacteria and yeasts isolated from maple sap.

To obtain a measure of the storage stability of irradiated sap, synthetic saps containing 1.1 x 10^5 bacteria per ml were irradiated for 8.0 sec (flow rate of 10.5 gal per min and two units). This sap, after irradiation, contained 3.1 x 10^2 organisms per ml. Two, 2.5 gal portions of the irradiated sap were stored in sterile carboys at 40 F and 70 F, respectively. Bacterial counts were made at 24-hr intervals. After 3 days' storage at 40 F, the bacterial count had increased to only 6.2 x 10^3 cells per ml. However, at

the higher storage temperature (70 F), the bacterial count increased 1000-fold during 24-hr storage. This suggests that irradiated sap can be stored without further treatment for considerable periods of time providing: 1) the bacterial count is reduced to a low level, e.g. 3 x 10^2, and 2) the storage temperature is 40 F or below.

SUMMARY

1) A method has been developed for controlling or reducing the number of bacteria and/or yeast in maple sap.
2) The method utilizes ultraviolet radiation in the region of 260-270 mμ using commercially available equipment and 30-watt ultraviolet lamps.
3) The method is a continuous process, with the sap flowing in a ½-in thick layer through an irradiated zone.
4) The effectiveness of the ultraviolet irradiation on reduction of bacteria and yeast is a function of exposure time. Exposure time = $\frac{\text{volume of sap in exposed area}}{\text{rate of flow}}$
5) Bacterial counts in sap can be reduced from 7 x 10^5 to less than 5 x 10^3 per ml with 8.5 sec of exposure.
6) Yeast counts in sap can be reduced from 7 x 10^3 to 1 x 10^2 per ml with 8 sec of exposure.
7) Microbial contaminated maple sap, after irradiation, can be stored several days at temperatures up to 40 F without any deterioration due to microbial fermentation.
8) Since the microbial control is effected by physical means, there is no chemical residue problem.
9) The ultraviolet irradiation of sap produces no alteration or noticeable effect on the sap or the syrup made from the sap.
10) The ultraviolet irradiation of sap in no way alters the amount or the quality of the maple flavor produced in syrup made from irradiated sap.

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